

Dynamic changes of phenolic compound contents in leaf and bark of poplar during autumn temperature drop

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Abstract: We investigated seasonal dynamics of phenolics substance in leaf and bark of two cultivars of poplar, *Populus alba* × *berolinensis* and *P.* × ‘Zhonglin Sanbei 1’, during autumn temperature drop for analyzing the roles of phenolic secondary metabolites in cold resistance. Results show that the contents of condensed tannin and flavonoid in poplar leaf and the flavonoid contents in bark of *P.* × ‘Zhonglin Sanbei 1’ were increased with the decrease of autumn minimum temperature, showing a significantly negative correlation between the contents of soluble phenolic substance in the leaves and changeable temperature. In contrast, lignin content in the poplar leaves is decreased in the process of temperature drop, showing a significantly positive correlation. These results indicate that the variation in phenolic substance has a close correlation with its cold-resistance during the autumn temperature drop.

Key words: poplar; natural temperature drop; condensed tannin; flavonoid; lignin

Introduction

Phenolic substance is one type of secondary metabolites synthe-

sized widely in higher plants with the structure of phenolic hydroxyl group and the related structure. This type of metabolites can be classified based on its structure to several subclasses including lignin, tannin, phenolic glycoside, and flavonoid, etc. (Chen and Ye 1998). In general, phenolic secondary metabolites play important roles in protecting plants from diseases and insect pests, bacteriostasis, and feeding (Walton 1996; Agrawal 1998; Dixon and Ferreira 2002; Morimoto et al. 2002; Teklemariam and Blake 2004). They are also efficient plant protectant against adverse environmental factors such as ultraviolet radiation, high light and atmospheric pollutants (Keinanen et al. 1999). Moreover, some researches showed that the dynamic changes of phenolic substance have close relations with plant resistance to low temperature (Li and Sakai 1982; Fei et al. 1994; Hoshino et al. 1999; Priyanka et al. 2001). Polar is an important afforestation and greening tree species in Northern China; however, this species is often subjected to cold injure, which become a main problem in forestry production and planting. In recent years, to obtain the fast-growing and high-yield plantation, some south cultivars are increasingly introduced into polar breeding and planting. In the combination of frequent abnormal climates, poplar trees are subjected to more heavy cold injures.

Li (2007) did some work on the seasonal dynamics of total phenol, flavonoid, condensed tannins, phenolic glycoside, and lignin in polar leaves. He found that there were significantly negative correlation between soluble phenolic substance (condensed tannin and flavonoid) of the leaves and minimum temperature, and positive correlation between lignin and minimum temperature. Moreover, significantly negative correlation was found between flavonoid/lignin contents and minimum temperature. Gao et al. (2009) investigated dynamics of phenolic compounds of poplar cutting branches in artificial temperature decrease, but no significant change was found. In the present study, we measured the contents of flavonoid, condensed tannin, and lignin in the samples in the process of natural temperature drop. The aims of this study were to test the relation between phenolic dynamics of polar leaves and temperature change and to analyze the roles of phenolic secondary metabolites in cold resistance.

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Materials and methods

Experimental materials

Populus alba × *berolinensis* (YZY) is an artificial crossing male clonal poplar. YZY has dense leaves and branches, green leaf epidermis, and white-hair back. The cultivar grows fast and has a high tolerance to drought and salt. Thus it is widely used for street trees, shelter belt, and planting trees in northeastern China (Zhang et al. 1994). *Populus* × ‘Zhonglin Sanbei 1’ (YC5) is a superior clone breeding selected from *P. nigra* × *P. simonii* mixed clones that was introduced by Mudanjiang forest in 1982 from Academy of China Forestry. This species has various advantageous features, characterized by fast growth, good quality in timber, straight form, narrow crown, thin branching, strong cold-resistance and disease-resistance, etc. Currently, the total planting area in Heilongjiang and Jilin provinces has reached more than 10×10^5 ha (Li et al. 1998).

Study area

The site is located in Harbin City (44°04′–46°40′ N, 125°42′–130°10′ E), Northeastern China. This region belongs to a continental monsoon climate, with typically alternating seasons. The autumn is a transitional season that air temperature is transferred from warm to cold. Seasonal average temperature is 9.4°C, and annual change of temperature ranges from 7.9°C to 11.1°C.

Sampling method

Street trees grown in two main streets of Harbin were selected. Five individuals of YZY (8-year old, diameter at breast height of 9.3–10.8 cm) and YC5 (7-year old, DBH 7.6–8.3 cm) with the same growth status were selected randomly, respectively; two 2-year-old branches in the crown layer of the down of the middle part of each polar tree were collected. Sampling was conducted from October 1 to November 3 (full defoliation), 2009 at the interval of three days. Sampling time was set at hour 9–10 a.m.. All leaves and barks of two-year-old branches in the same tree were collected as one sampling. Sampling fixation was carried out at 105°C for 30 min. After that, the samplings were dried at 60°C for 72 h, crashed, and sieved for further use.

Experimental methods

Extraction of flavonoid and condensed tannin referred to the method of Li (2007). Visualization method and assaying conditions of flavonoid, condensed tannin, and lignin referred to Xi and Li (2000), Butler et al. (1982), and lignin Wei et al. (2001), respectively.

Temperature change in Harbin during sampling period

For investigating the relation between phenolic dynamics of

polar leaves and temperature change, we analyzed temperature data of the site during sampling period, and averaged the minimum temperature for three days before the first sampling date in 2009. Analysis results showed that the minimum temperature was decreased gradually from 10°C to -8°C, with a monthly decrement of 18°C (Fig. 1).

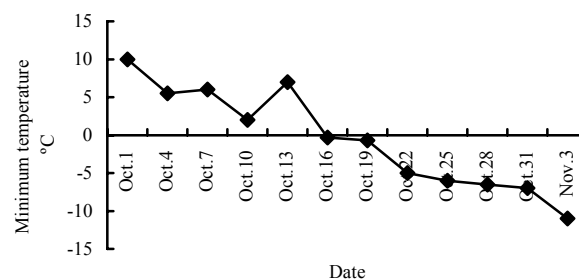


Fig. 1 Variation in minimum temperature in Harbin during October in 2009

Results

Dynamics of leaf phenolic substance

Condensed tannin

Condensed tannin content in poplar leaf showed an increasing trend with temperature drop in October, with an increment of 2.48 times for YC5 and 1.0 times for YZY (Fig. 2a). In contrast, the content of condensed tannin in polar barks had no significant change during the sampling period, although it had a large fluctuation.

The content of condensed tannin in leaf and branch bark of YZY was significantly lower than that of YC5 ($p < 0.01$). For cultivar YC5, condensed tannin in leaf was significantly lower than that in branch bark at the first sampling, but it showed an opposite pattern at the last sampling. For cultivar YZY, the content of condensed tannin in leaf was higher than that in branch bark (Fig. 2).

Flavonoid

Flavonoid content of leaf showed an increasing trend with temperature drop in October for both YZY and YC5, with a large fluctuation in YZY and small fluctuation in YC5. Flavonoid content in leaf was increased by 44% for YC5 and 81% for YZY during October. There was no fluctuation in bark flavonoid for both polar species.

Flavonoid contents in leaf and branch bark of YZY were lower than that of YC5. Flavonoid content in leaf was significantly higher than that in bark ($p < 0.01$) for both species. Moreover, although flavonoid content in YZY leaf was lower than that of YC5, the flavonoid increment in YZY leaf was significantly higher than that of YC5 leaf during autumn temperature drop (Fig.3).

Lignin

Lignin content in polar leaf decreased with temperature drop in October (Fig. 4a), showing an opposite pattern as compared to phenolic substances. Lignin content in leaf was decreased by 23% for both YC5 and YZY. The variation of lignin content in

branch bark showed a similar trend with leaf.

Lignin content in leaf of YZY was significantly higher than that of YC5 ($p<0.05$), but the lignin content in bark showed an opposite pattern ($p<0.01$). In general, lignin content in leaf was higher than that in bark for both poplar species ($p<0.01$) (Fig. 4).

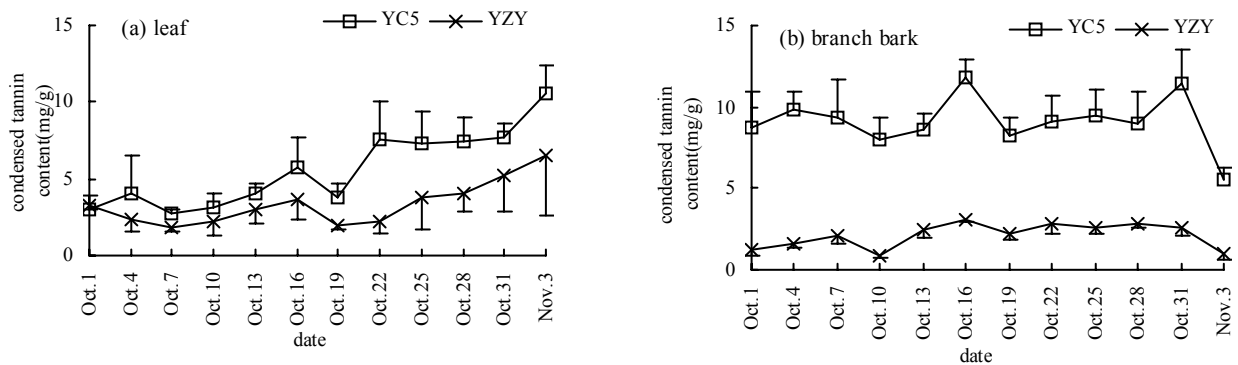


Fig. 2 Variation in condensed tannin in poplar leaf (a) and branch bark (b). YC5---*P. nigra* × *P. simonoi*, YZY---*Populus alba* × *berolinensis*

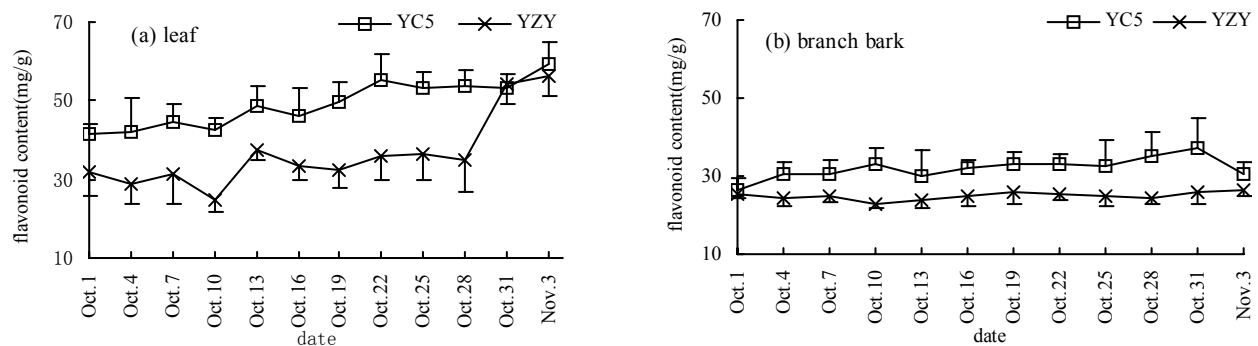


Fig. 3 Variation in flavonoid contents of poplar leaf (a) and branch bark (b). YC5---*P. nigra* × *P. simonoi*, YZY---*Populus alba* × *berolinensis*

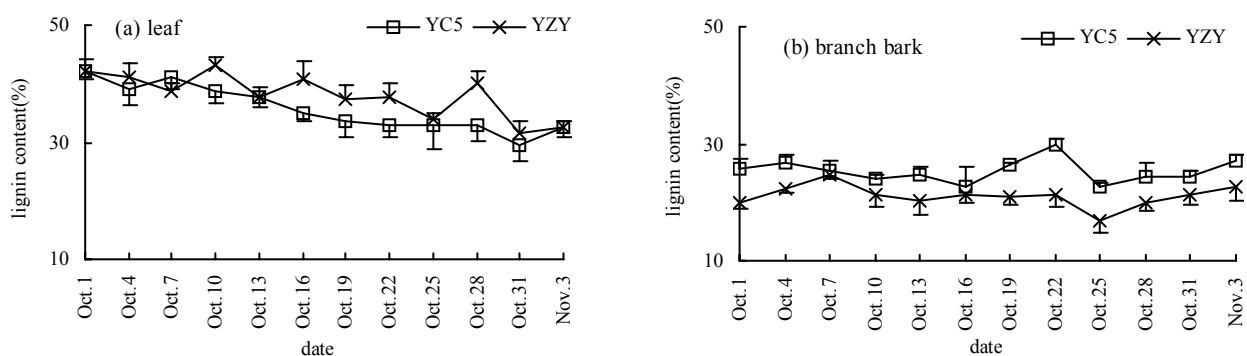


Fig. 4 Variation in lignin contents of poplar leaf and branch bark. YC5---*P. nigra* × *P. simonoi*, YZY---*Populus alba* × *berolinensis*

Correlation analysis of phenolic content and minimum temperature

For both YC5 and YZY, condensed tannin and flavonoid in leaf had a significantly negative correlation but lignin content in leaf

had positive correlation with minimum temperature. There was significant negative relation between flavonoid in bark and minimum temperature for YC5 but not the case for YZY (Table 1).

Table 1. Correlation between dynamics of phenolic substance and minimum temperature

	Phenolic pounds	com- coefficient	Correlation coefficient	Significant (<i>p</i>)	Sample number (N)
YC5	Condensed tannin		-0.904**	0.000	12
Leaf	Flavonoid		-0.891**	0.000	12
	Lignin		0.923**	0.000	12
YZY	Condensed tannin		-0.604*	0.038	12
leaf	Flavonoid		-0.641*	0.025	12
	Lignin		0.695*	0.012	12
YC5	Condensed tannin		0.105	0.745	12
branch	Flavonoid		-0.678*	0.015	12
bark	Lignin		-0.060	0.853	12
YZY	Condensed tannin		-0.373	0.232	12
branch	Flavonoid		-0.422	0.172	12
bark	Lignin		0.223	0.486	12

YC5---*P. nigra* × *P. simonii*, YZY---*Populus alba* × *berolinensis*

Discussion

Plant growth is regulated by itself hereditary substance. Environmental factors significantly affect plant growth partially by regulating genetic expression and protein synthesis (Pan 2001). Plant secondary metabolites are the complex products of interaction between plant and environment (biological and abiological factors) in a long-term evolution (Massei et al. 2000). These metabolites play important roles in the improvement in itself protection, survival competition capability, and the coordination with environment. There is more close relationship between the yield and change of secondary metabolites and environment than that of original metabolites (Gershenson 1984; Shelton 2000). Phenolic species are the main components of secondary metabolites in poplar trees, which vary with environmental factors.

Li (2007) did some work on seasonal dynamics of poplar phenolic substance. He found that during autumn with temperature drop, condensed tannin in poplar leaf was increased by 186% for YC5 and 311% for YZY5, flavonoid content in leaf was increased by 73% for YC5 and 131% for YZY5, and that lignin content in poplar leaf decreased by 14% for YC5 and 23% for YZY5. In the present study, we found that the condensed tannin content in poplar leaves was increased by 248% for YC5 and 100% for YZY during the autumn with temperature drop, the flavonoid content in leaves was increased by 44% for YC5 and 81% for YZY, and that lignin content in leaves was decreased by 23% for YC5 and 23% for YZY. These findings are agreed with that result reported by Lindroth et al. (2002) and Lindroth and Hwang (1996).

With the continuous decrease of temperature, soluble phenolic substances (flavonoid and condensed tannin) in YC5 and YZY leaves were negatively correlated with temperature decrease at the level of significance and/or extreme significance. Flavonoid content in YC5 bark was significantly increased with the decreasing temperature. This implies that increasing soluble phenolic substances in the leaves may be linked to cold resistance

when temperature is dropped sharply. Condensed tannin in branch bark maintained at a higher level compared with that in the leaf, although the content in YC5 bark was not increased significantly with the decrease in temperature. Thus it may explain why cold-resistance of YC5 is superior to YZY.

Soluble phenolic substances in YC5 leaves were significantly higher than that of YZY in the original value measured on Sep. 30. However, the increment in YZY leaves was larger than that of YC5 leaves with the decrease of temperature, indicating that YZY had a stronger response to the decrease in temperature. It implies from the other hand the important role of soluble phenolic substances in polar cold-resistance.

Lignin and soluble phenolic substances that are secondary metabolites synthesized in shikimic acid pathway have close correlation with plant growth and stress resistance (Li et al. 2003). Our study showed that lignin content in poplar leaf had positive correlation with temperature drop; in contrast, there was significantly negative correlation between the soluble phenolic substances and temperature change. Therefore, it is assumed that the increasing soluble phenolic substances might be transformed from the degradation of lignin. It also indicates that mutual transformation of soluble phenolic substances may play an important role in poplar cold-resistance.

Gao et al. (2009) conducted an experiment to study the response of poplar cutting branches to abrupt temperature decrease for 2 h. They found that there was no significant change in phenolic compounds in polar leaves; thereby they inferred that the temperature treatment for such a short-term is inadequate for the synthesis of phenolic compounds. However, our study showed that long-term natural temperature decrease induced a large accumulation of phenolic compounds in the poplar leaves.

During sharp temperature drop in autumn, both leaf soluble phenolic substances (condensed tannin and flavonoid) of poplar trees and flavonoid content of YC5 bark were increased with decreasing temperature. In contrast, leaf lignin content was decreased with decreasing temperature, with a significantly positive correlation with minimum temperature. During natural temperature drop, the changes of poplar phenolic substance might be correlated with cold-resistance.

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